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Capt. Steve Lewis, MC, USN

Department of the Navy

Naval Medical Research and Development Command

Combat Casualty Care Research

Naval Medical Center

Bethesda, Maryland 20814-5044

Status report covers progress made through the period

October 1, 1991 through March 31, 1992

on N00014-89-J-3124

P.I. Harvey I Miller, Ph.D.

Metabolic Changes and Hemodynamic Dysfunction

Following Hypothermic Shock

Grant Number: N00014-89-J-3124

Harvey I Miller, Ph.D. - Principal Investigator

STATUS REPORT

From October 1, 1990 To March 31, 1992

When the entire organism is chilled in a very cold environment, the temperature regulatory system is overwhelmed and the core temperature starts to fall rapidly. When the core temperature falls below 35°C, the individual is believed to be hypothermic. Accidental hypothermia occurs so rapidly that the body's physiologic functions slow down and little metabolic or mechanical reactions can occur. Little damage is found during the cold stages of hypothermia. Extreme vasoconstriction of peripheral tissues occurs, and what metabolism occurs is anaerobic. These tissues produce lactic acid, which stays in the tissue due to lack of circulation. From the moment the body is exposed to the cold, large quantities of catecholamines are released from the adrenal medulla. In the guinea pig it is mostly norepinephrine. The metabolic consequence of the catecholamines is only seen early before the body temperature falls below some critical point and chemical reactions are slowed.

We have shown in a guinea pig model that following cold water immersion, hypothermia was produced, and following rewarm, a cardiac dysfunction was observed which persisted for at least 48 hours. Cardiovascular compensatory

mechanisms overshadowed the dysfunction and it was not readily apparent in the whole animal. However, if the hearts from hypothermic animals are isolated from neural and endocrine influences by using the perfused working heart preparation, the hypothermics exhibit depressed Starling curves. In other words, hearts from hypothermically shocked animals who were rewarmed have decreased physiologic reserve. If the animal's physiologic reserve is low when it is placed in the cold environment, then the animal may not survive. We use a Duncan-Hartley strain of guinea pig, who have an absolute requirement for Vitamin C. We have found that if the animals get their ascorbic acid from greens instead of in their Purina chow, they do much better and appear to be more healthy. Due to a mixup our animals did not get greens for a substantial amount of time. Since this brought a decrease in their physiologic reserve, we have discarded all data from this time.

Progress was made in measuring plasma catecholamines in the cool down and rewarm phase of hypothermia.

Guinea pigs who were fed leafy greens for at least 2 weeks prior to any experiment had indwelling catheters and a thermistor emplaced as described in earlier progress reports. On the day of the experiment, they were temporarily anaesthetized with a short-acting barbiturate, Brevital®, and immersed neck-deep in ice-water until their core temperature fell to 25.5°C. This process took from 6-7 minutes. Observations were recorded and some blood samples taken at 30 seconds after immersion and then after every minute. When the core temperature reached 25°C, the animal was removed, dried and rolled in a heating pad. Even though the guinea pig was being warmed, during the first few minutes the body temperature

continued to fall. When it reached its lowest point (LBT), blood samples were withdrawn and recordings of heart rate, blood pressure and body temperature were obtained. The body temperature began to slowly rise and when it reached 38.5°C some 45 minutes later, blood samples plus previously mentioned parameters were taken. This point was considered zero (0) time. Recordings and blood samples were also taken at 1, 2, 3, 4 and 24 hours.

Figure 1 shows the changes in body temperature from control samples, through the cool down, rewarm and 1, 2, 3, 4 and 24 hours after return to normal temperature. We have expanded the time interval between control and lowest body temperature (LBT). It appears that at 1 minute body temperature is still being maintained, however, at 2 minutes all the compensatory mechanisms are overwhelmed and a significant drop in temperature is seen which continues to the LBT. The control animal, immersed in warm (38-39°C) water has just a small drop in body temperature over the 7-8 min. The temperature is restored with a few minutes after exposure to a heating pad.

Heart rate (HR) changes during cool down and warmup are shown in Figure 2. The lower figure is an expanded view of the time from the control sample to the lowest body temperature. Even though the animal is anaesthetized with Brevital®, the heart rate increases dramatically upon contact with the cold water. At 30 seconds it peaks and starts down. By 1 minute it is down by 10% and as the body temperature falls, the heart rate falls. It reaches its lowest point at the LBT. HR returns to its preimmersion at the same time that the body temperature returned. Cardiac output fell in both the control and experimental animals after anesthesia (Figure 3). However, the cardiac output control animals rose after immersion, while

that of the hypothermics fell. The cardiac output in the hyperthermic animals began to rise 1 hour after return to preimmersion temperature. At 4 hours, control animals still did not have a normal cardiac output. It wasn't until 24 hours later that cardiac output was at preanesthesia levels for both control and hypothermic.

During cool-down, plasma norepinephrine concentration (NE) steadily rose in hypothermics while the control levels were constant (Figure 4). At 4 minutes post immersion, when the body temperature reached 25 °C, NE is 3-fold that of the control. In 2 hours NE is still much higher than the control. By 3 hours the levels are the same as controls. However, 48 hours after return to normal body temperature, a difference is seen again. Epinephrine (E) levels are very varied; however, between 0-3 hours E values are elevated.

With the high norepinephrine levels one would expect the free fatty acids (FFA) to be elevated, but we have not observed that (Figure 6). While the blood glucose levels of both the hypothermic and control animals rise during cool down, they return to normal values at 0 time in the controls, and fall to low levels at 2 hours post return to normal temperature, reflecting insulin release (Figure 7). By 4 hours they are essentially normal. The hypothermic FFA levels peak at 0 time. However, by 4 hours they are normal. Lactate rises steadily in the hypothermic and controls. The elevation of control lactate levels, while statistically significant, are slight, while the elevation in hypothermics is large, reflecting ischemia in the peripheral tissues with wash out or rewarm (Figure 8).

These metabolic studies suggest that while there are very large increases in circulating catecholamines, the catecholamines are less sensitive in the hypothermics post rewarm. Heart rate is elevated almost immediately upon immersion in cold

water and does not fall until 2 minutes after immersion. When the body temperature returns to normal (0 time) so does the heart rate (Figure 2), even in the face of high catecholamine levels. It would appear that the adrenergic receptors are downregulated and appear less sensitive.

There may be another explanation for the lack of elevation of the FFA. The "Adipose Tissue Electric Blanket Theory" suggests 2 roles to the subcutaneous adipose tissue. 1) It is a very good insulator and preserves the heat in the core. 2) The cycling of triglyceride, the major component in adipose tissue, is hydrolyzed to 3 FFA and glycerol and then reesterified into triglyceride. The product of this cycle is heat. Hormone sensitive lipase controls this cycle. Norepinephrine controls hormone sensitive lipase activity. The reason for the lack of elevation of plasma FFA might be an increase in the internal cycling of fatty acids to produce more heat. However, the hypothermic animals' adrenergic receptors appear to be downregulated and these animals have a difficult time maintaining core temperature even 4 hours after rewarm. It is more than likely that the hormone sensitive lipase is depressed, the futile cycle is slowed, and heat production within the adipose tissue is depressed.

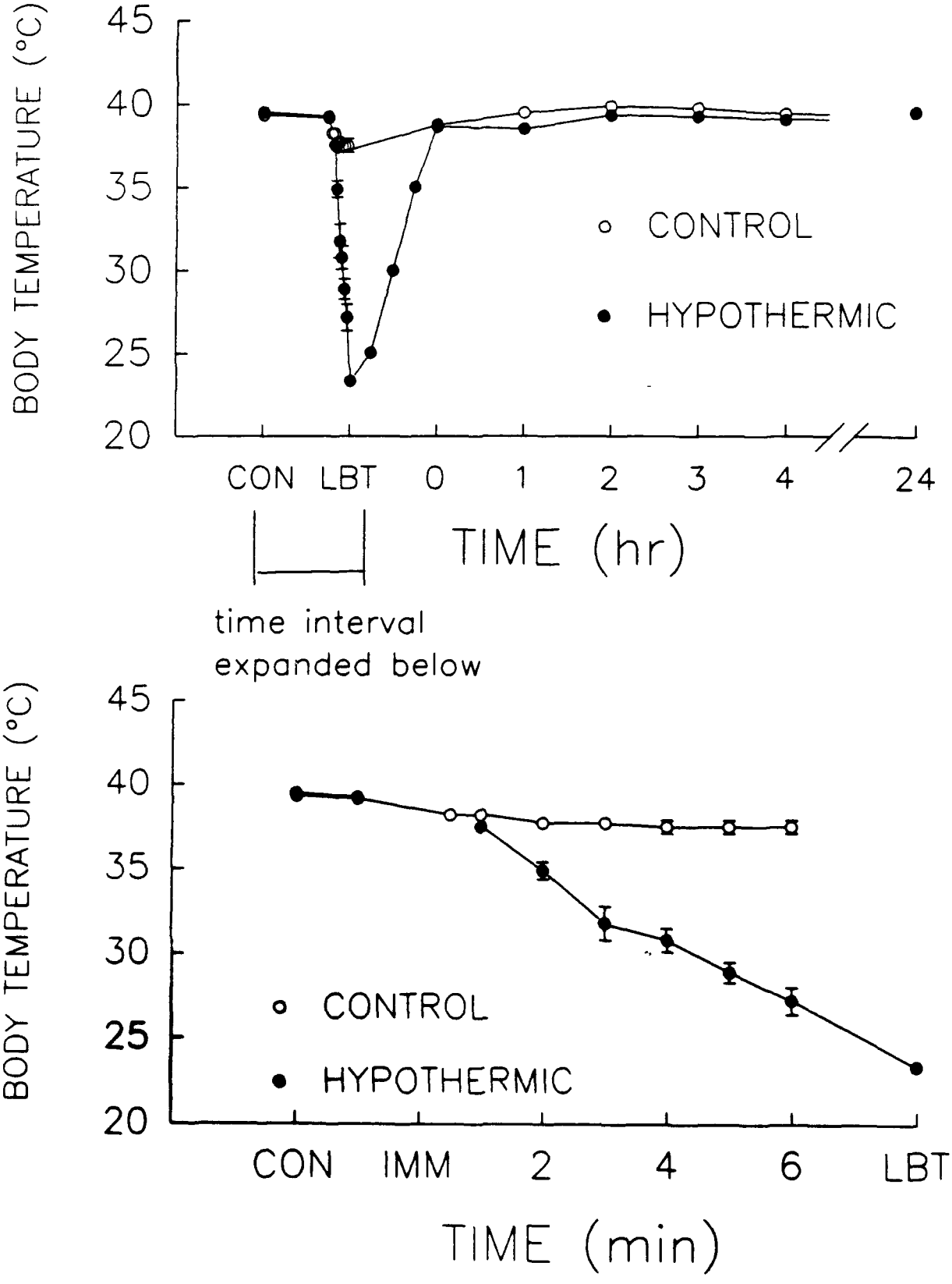
In order to study these phenomena we are measuring both plasma FFA and glycerol to determine the retention of FFA by adipose tissue. Since the freed glycerol cannot be rephosphoralated within the adipose tissue, glycolysis must supply the α glycerophosphate, so that the ratio of glycerol to FFA in the plasma is a reflection of adipose tissue reesterification. In this way we can tell whether the loss of temperature regulatory ability is due to a short circuit of the electric blanket.

The changes we observed in the metabolic samples (FFA, glucose and lactate) are related to the elevation of NorEpi. When the energy baring metabolites cannot

be mobilized by NorEpi due to downregulation and the futile cycle of triglycerides within the adipose tissue does not produce the heat required, then the organism can no longer thermoregulate.

NAVAL6.WP

FIGURE 1

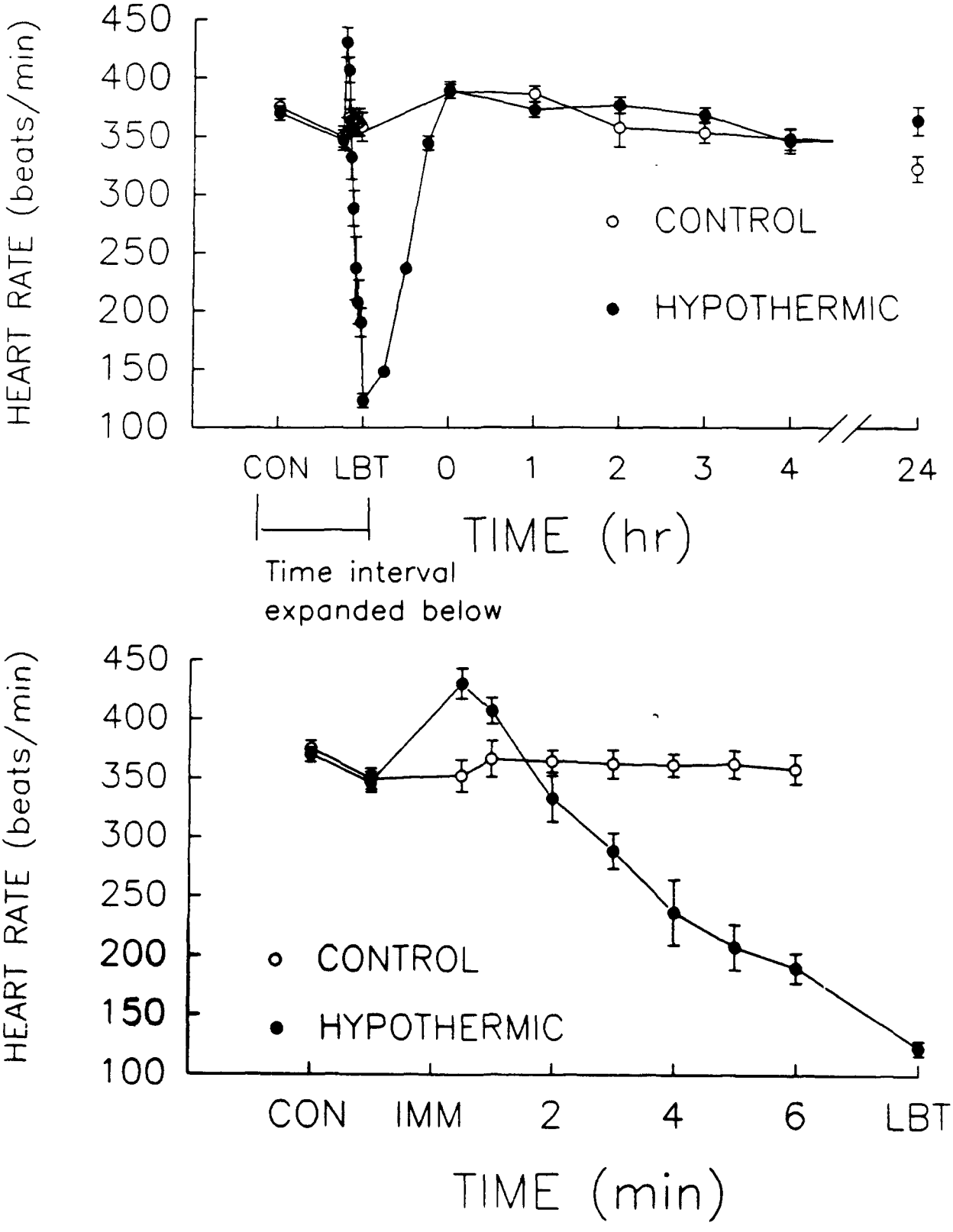


CON– CONTROL

IMM– IMMERSION

LBT– TIME OF LOWEST BODY TEMP

FIGURE 2

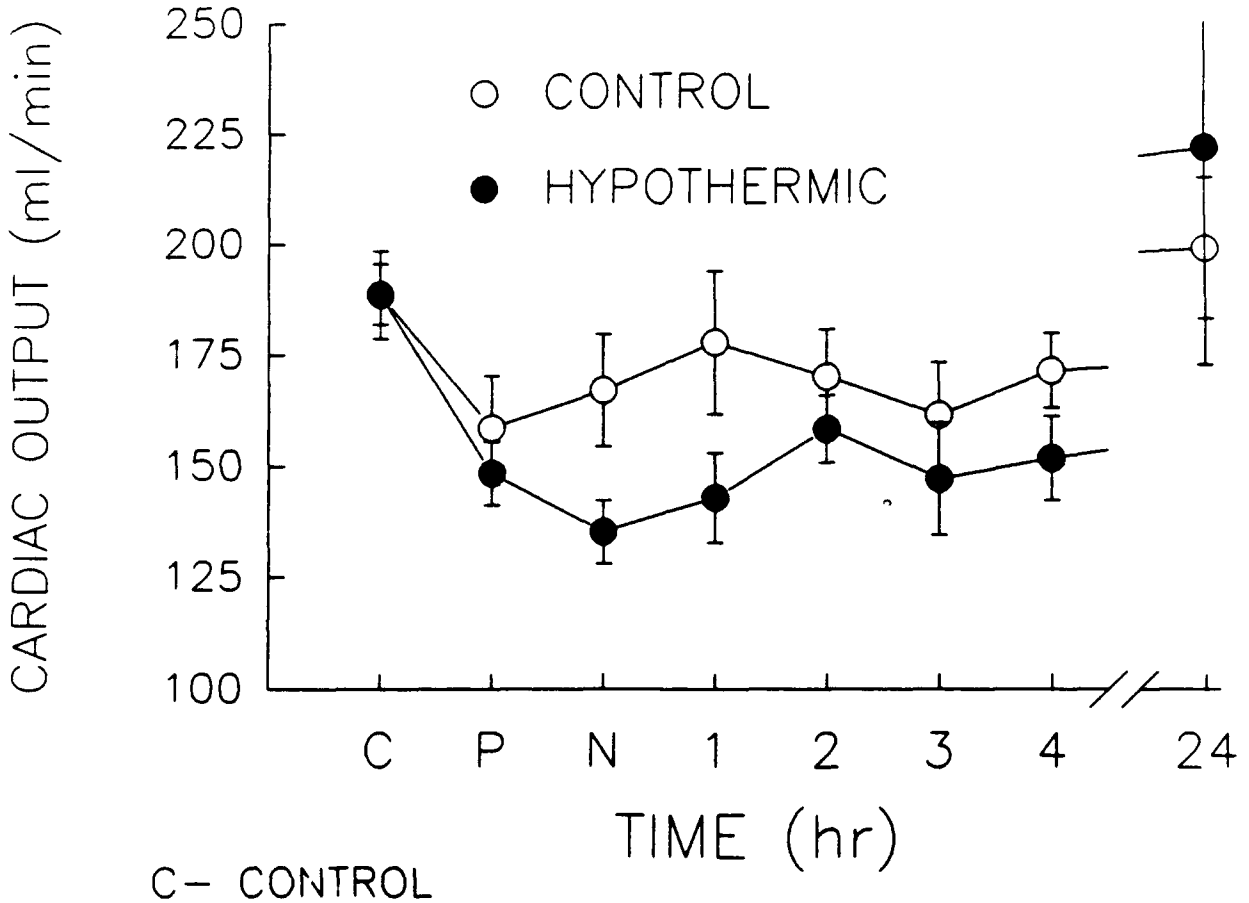


CON– CONTROL

IMM – IMMERSION

LBT – TIME OF LOWEST BODY TEMP

FIGURE 3

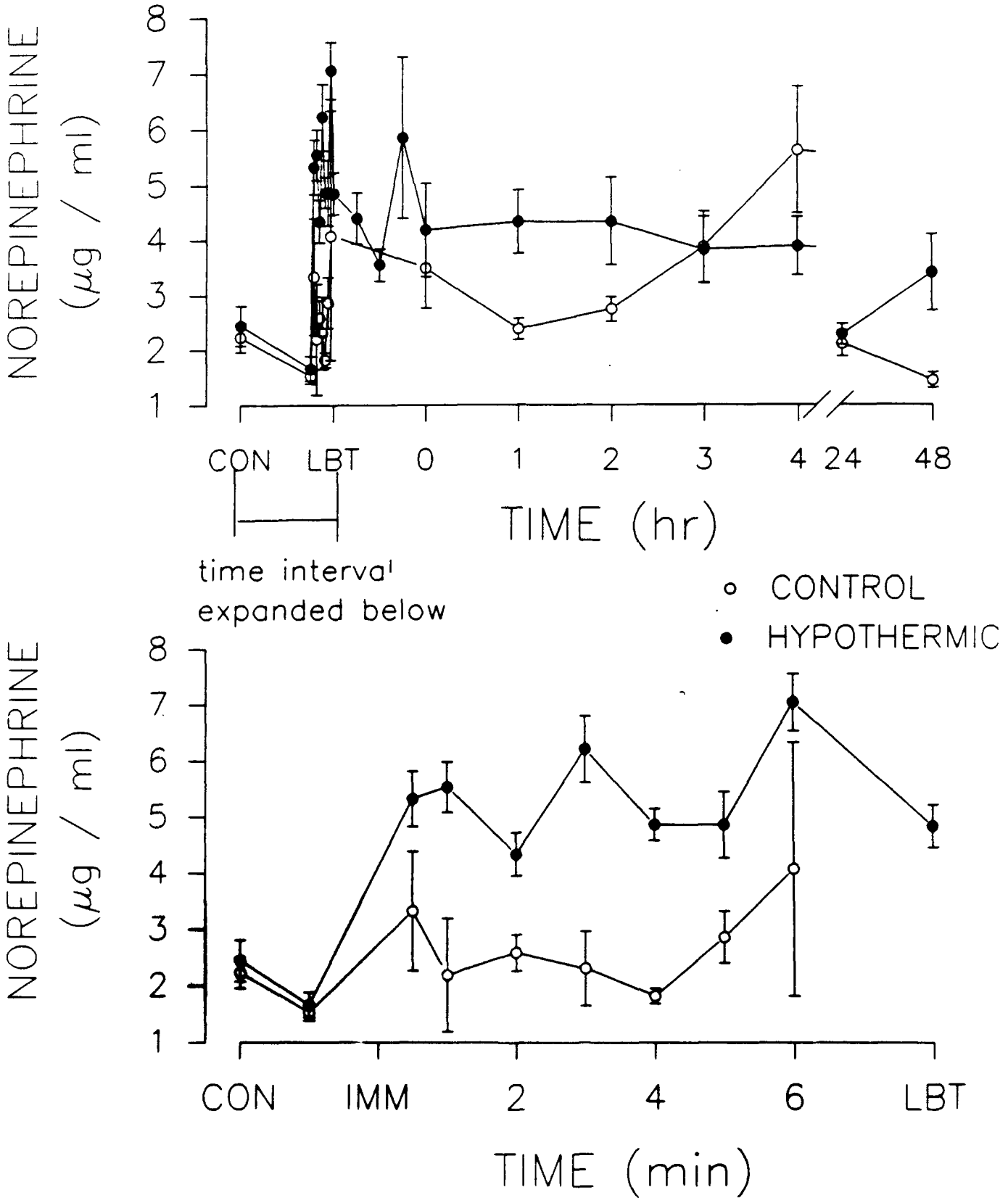


C– CONTROL

P– ANESTHETIZED PREIMMERSION

N– TIME OF NORMAL BODY TEMP – 0 HR

FIGURE 4

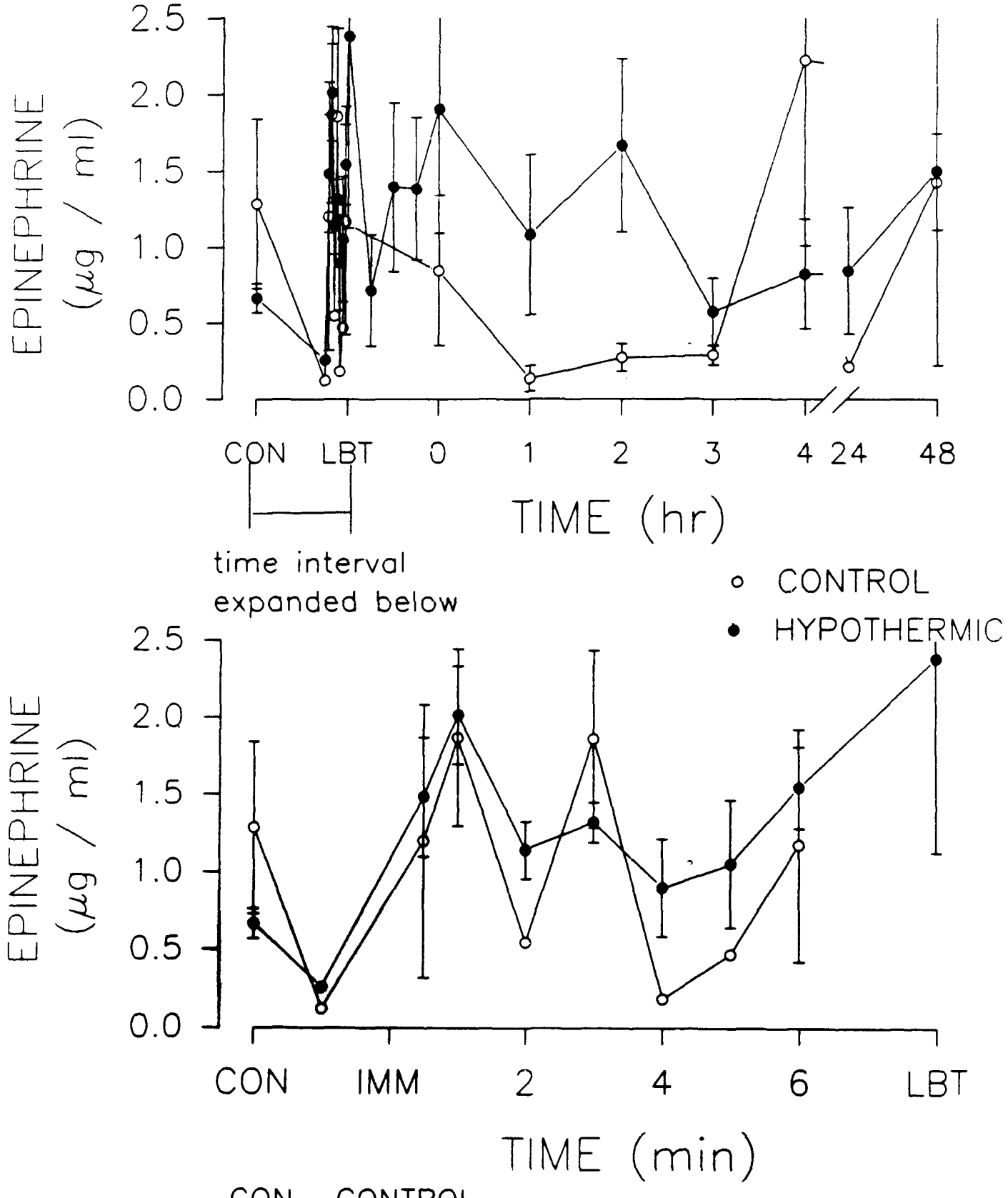


CON– CONTROL

IMM– IMMERSION

LBT– TIME OF LOWEST BODY TEMP

FIGURE 5

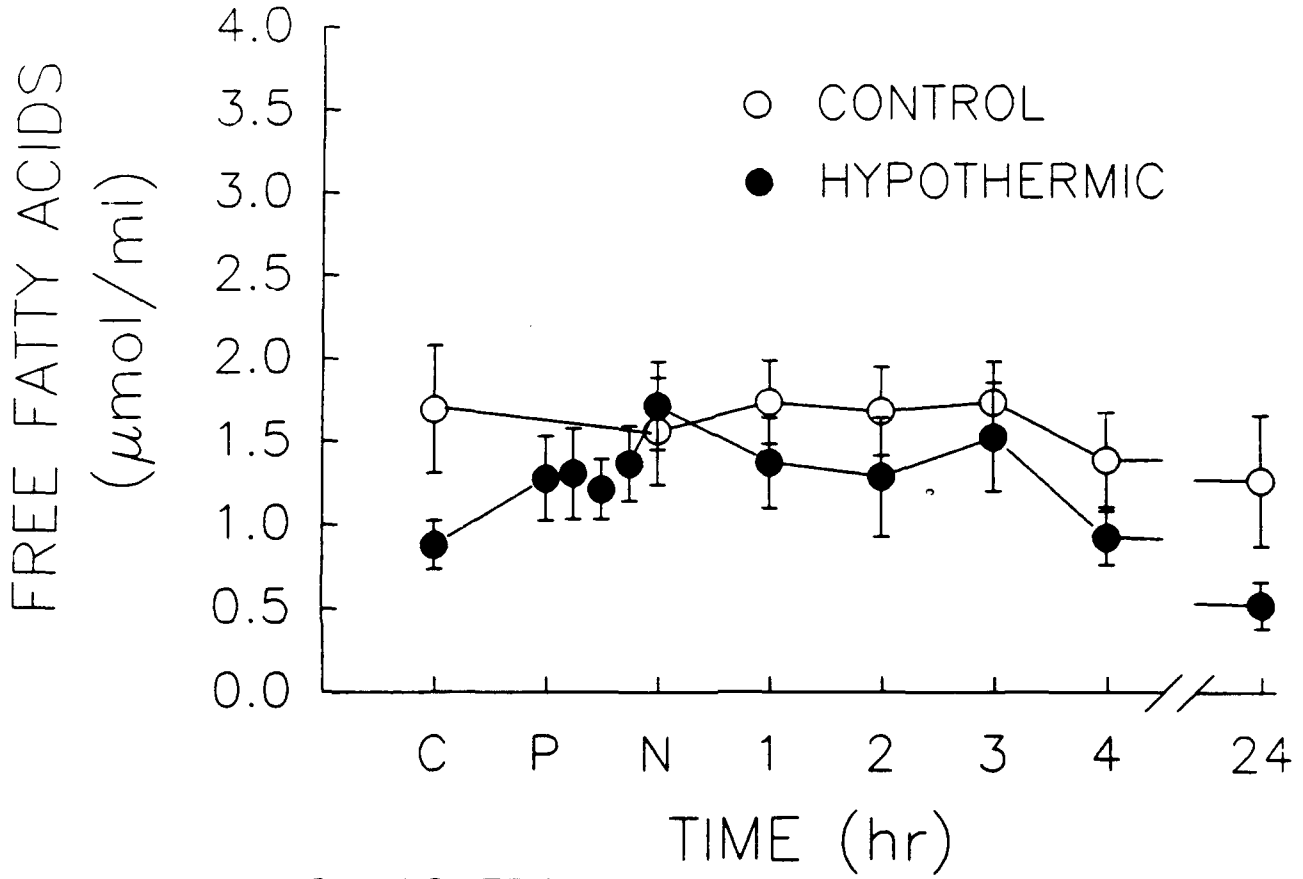


CON– CONTROL

IMM– IMMERSION

LBT– TIME OF LOWEST BODY TEMP

FIGURE 6

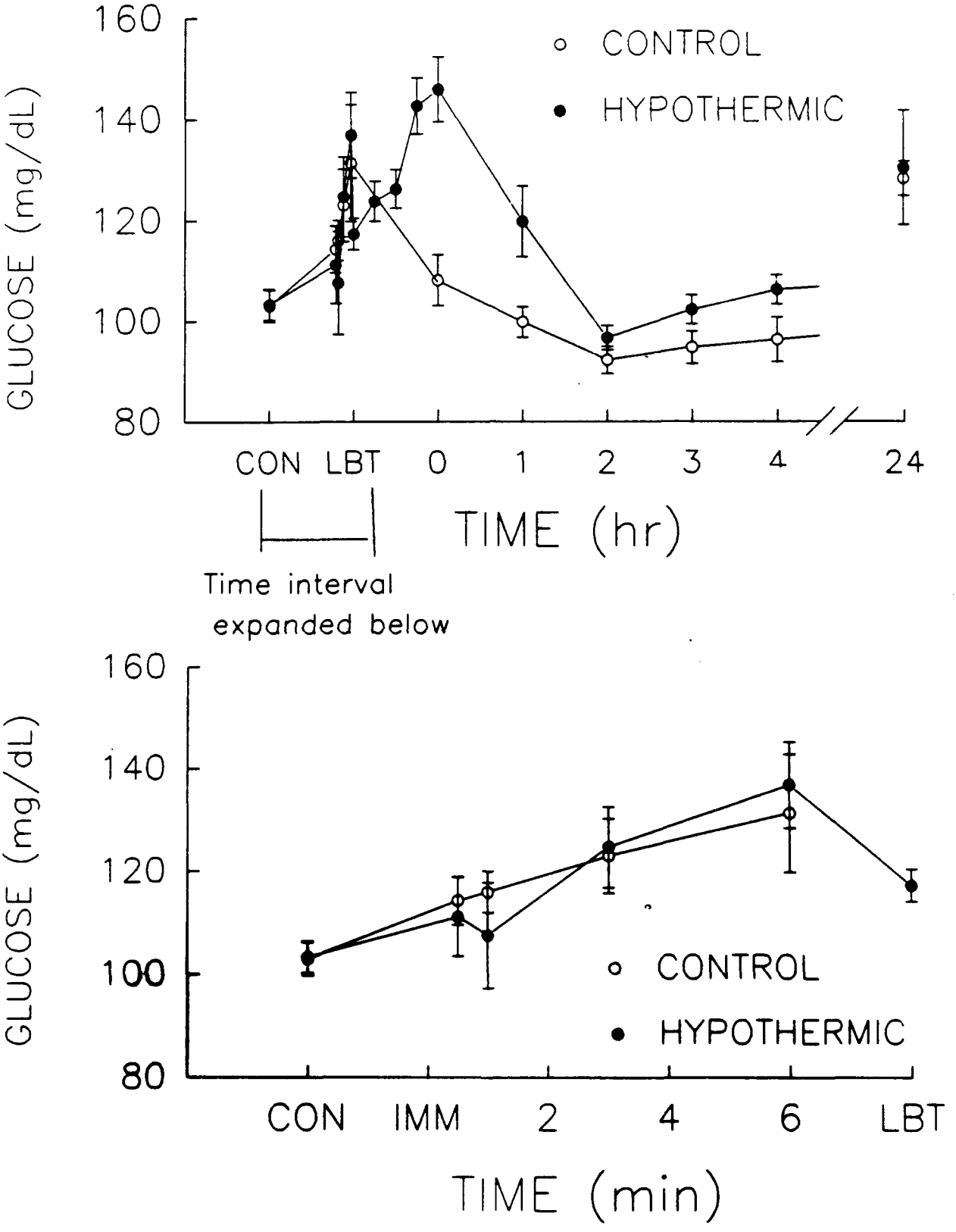


C– CONTROL

P– ANESTHETIZED PREIMMERSION

N–TIME OF NORMAL BODY TEMP – 0 HR

FIGURE 7

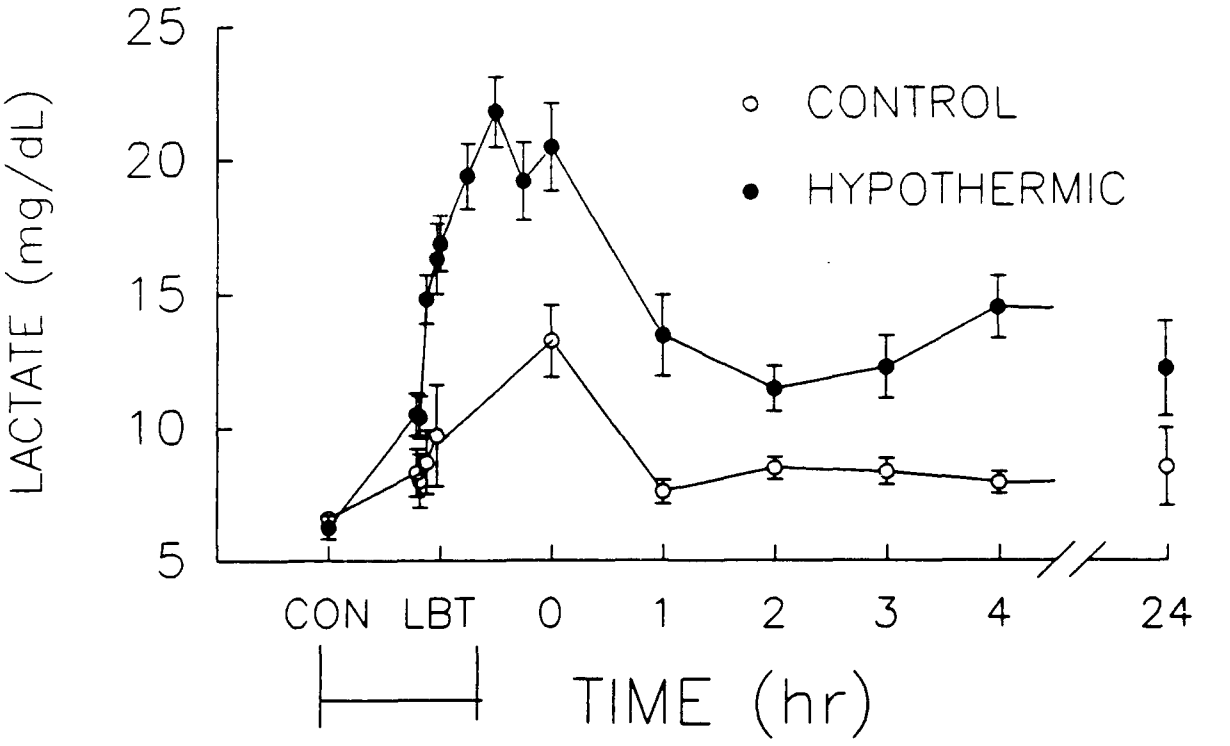


CON– CONTROL

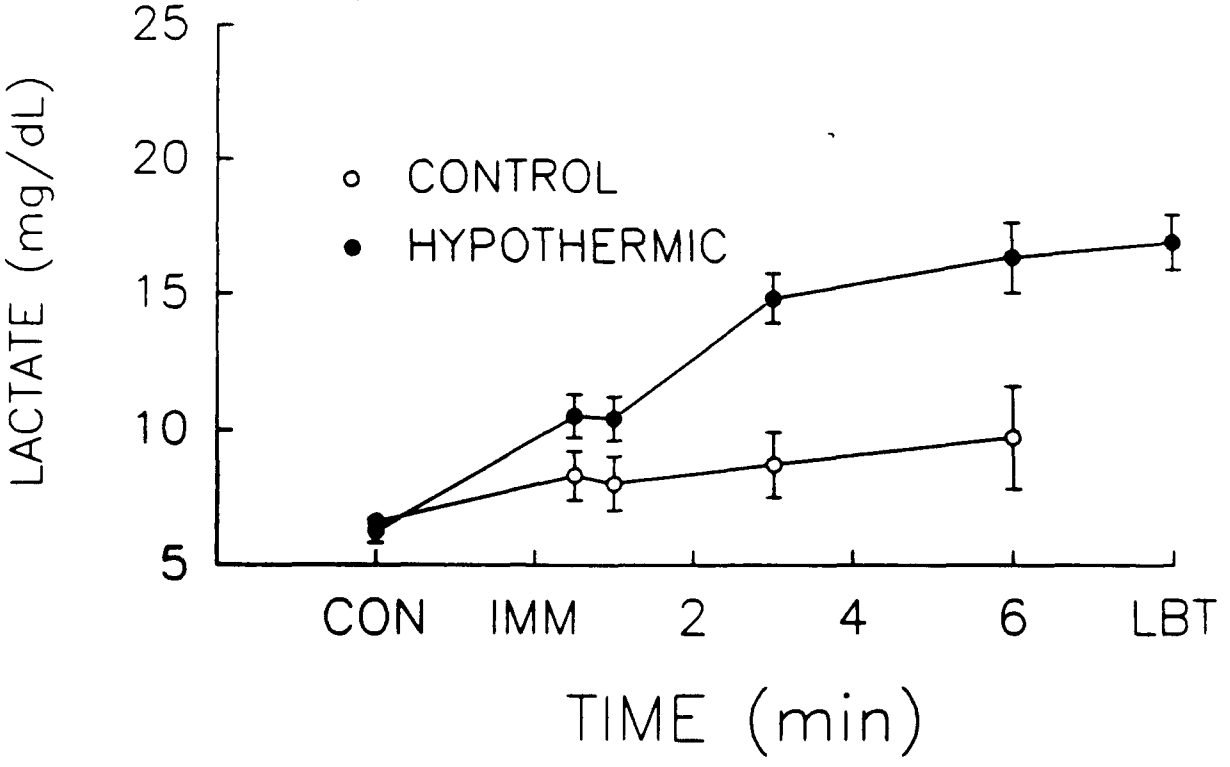
IMM – IMMERSION

LBT – TIME OF LOWEST BODY TEMP

FIGURE 8



Time interval expanded below

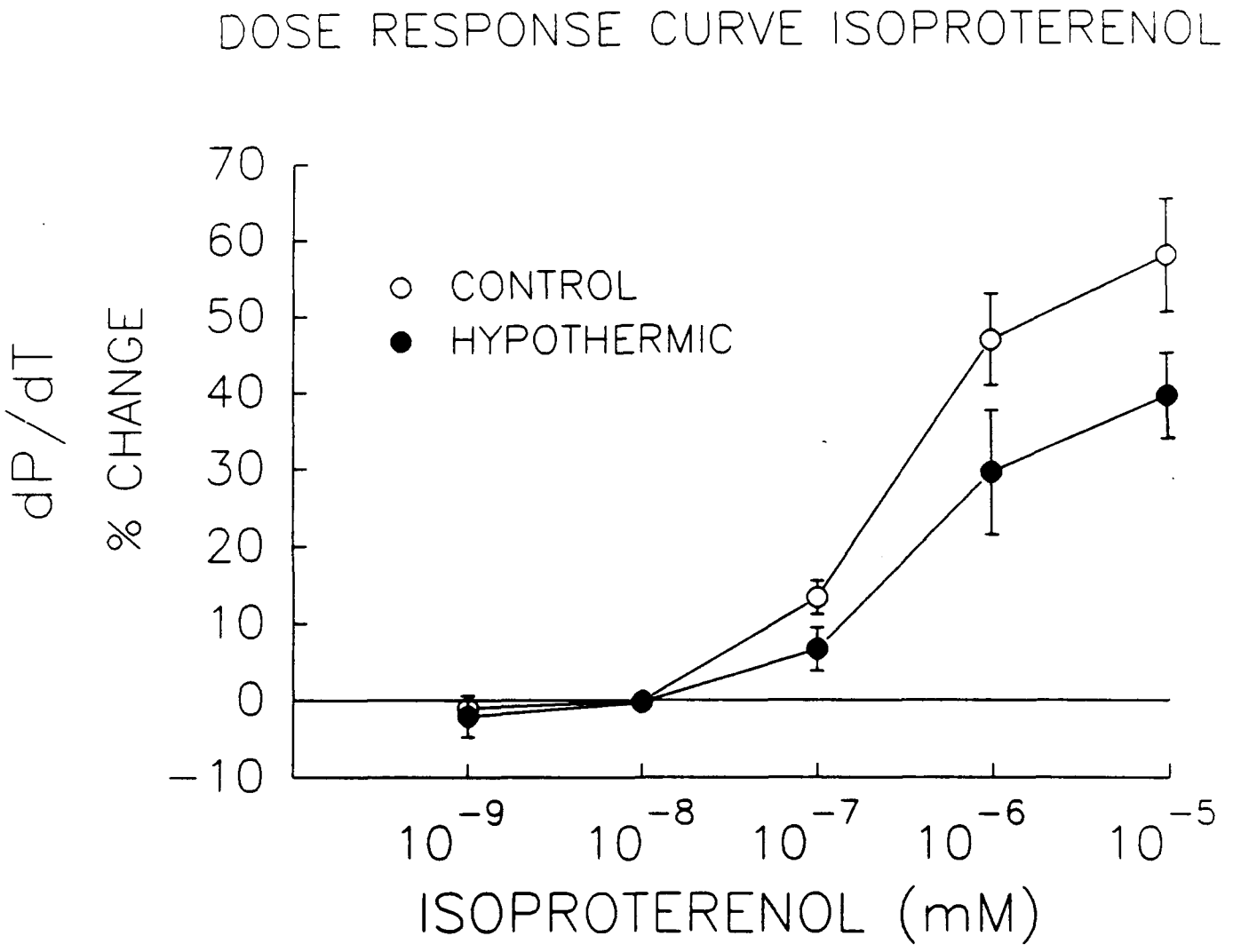


CON– CONTROL

IMM – IMMERSION

LBT – TIME OF LOWEST BODY TEMP

FIGURE 9


DOSE RESPONSE CURVE ISOPROTERENOL
dP/dT
% CHANGE
70
60
50
40
30
20
10
0
−10
CONTROL
HYPOTHERMIC
10^-9
10^-8
10^-7
10^-6
10^-5
ISOPROTERENOL (mM)